SOME ASPECTS OF HELMINTHIC INFECTIONS IN THE POND MURREL, CHANNA PUNCTATUS (BLOCH)

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A C K N O W L E D G E M E N T S

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Shabana Firdaus

(SHABANA FIRDAUS)

G E N E R A L I N T R O D U C T I O N

GENERAL INTRODUCTION

Among some of the most important problems confronting the modern fish culturist are the parasites and diseases of fish. Although diseases are more prevalent among such fishes, like salmon and trout, that are generally propagated under unnatural high stock density, other pond fishes are by no means free from such hazards.

The prevalence of disease is, to a large extent, dependent on the environment, and the more artificial the environment becomes the greater is the probability of infection (Davis, 1958).

The great majority of the infectious diseases of fishes are caused by either bacteria or protozoa. Aside from the bacteria, the only common plant parasites are the water fungi (Saprolegniaceae), several species of which may attack fishes, occasionally causing considerable mortality. Epidemics due to virus infections are not uncommon, but our knowledge of these diseases seems much limited. Because of the largely unpredictable nature of disease outbreaks and

their frequently serious pathogenic effects, diseases rank among the more important constraints to holding fish captive. The appearance of disease in fish, beside being attributed to the action of infectious agents, could also be related to more general environmental factors, including water quality, inadequate diet and stress (McVicar and Richards, 1981).

Modern epidemiology is in fact based on the premise that epidemic outbreaks are caused by an imbalance between the hosts, the pathogens or other disease agents and the environment. Aquatic cold blooded animals are much more affected by the environment than are the terrestrial homotherms. The outbreaks of various diseases of fish are, therefore, strongly affected by ecological factors (Snieszko, 1972).

A great majority of fishes carry heavy infection of parasites which could cause deterioration in the food value of fish and may even result in their mortality. Besides, a number of helminth parasites are transmitted to man only through fishes. In India although the problem of helminthiasis in sheep, goat and cattle has received considerable attention in the past, little attention seems to have been paid to this aspect in relation to fisheries. It is only recently that attention is being focussed to the proper

study of fish parasites and fish pathology and their control measures.

Detailed reviews on fish pathology with particular reference to disease have been made by Amlacher (1970), Reichenbach - Klinke (1974), Ribelin and Migaki (1975) and Roberts (1978).

An epidemic in brown and rainbow trout, caused by pleurocercoids of Diphyllobothrium in a west of England reservoir caused heavy losses. Incidence of infection increased with the age of the host and that mortality appeared to be related to the number of pleurocercoids present (Fraser, 1960). The pyloric caeca were the most common site of cysts but in heavy infestation other organs were also affected, namely, liver, gonad, surface of the swim bladder, spleen, etc. Harris and Hickey (1944) contended that trout were infected not through feeding on infected copepods but by feeding on sticklebacks.

Heavy mortality of young Salvelines fontinalis in 1957 and 1958 at a hatchery in La Tuque, Quebec, Canada, was caused by the migration of relatively large diphyllobothriid pleurocercoids giving rise to internal haemorrhage (Hoffman and Dunbar, 1961).

Evidence for the pathogenicity of the salmon poisoning fluke, Nanophyetus salmincola, to fish was also studied (Baldwin et al., 1967). Ward and Mueller (1926), who examined brook trout fry that had died in an Oregon hatchery during a severe epizootic, found a direct relationship between the degree of popeye (exophthalmia) and the number of parasites in the optic nerve. Regarding the symptomatic gross and histopathologic changes associated with the infections in fish they believed that these parasites caused exophthalmia. Simms (1933) reported heavy N. salmincola infection in young rainbow and brook trout that were dying in an Oregon hatchery.

Bennington (1951), while commenting upon the pathogenicity of these (N. salmincola) parasites, stated that the parasite may be quite pathogenic to young fish during the season of low water in streams having high population of infected snails. Farrell and Lloyd (1962) reported that rainbow trout were killed after laboratory exposure to infected snails. They observed that the degree of pathogenicity was directly proportional to the rate of accumulation of cercariae by fish.

Bennington and Pratt (1960) observed that local dermatitis developed in salmon where carcariae had penetrated. Wood and Yasutake (1956) studied the histopathology associated

with the metacercariae in naturally selected hatchery raised yearling coho salmon and concluded that an infected fish is weakened physiologically in practically every organ. Further, Millemann and Knapp (1970) observed the pathogenicity of the United States strain of N. salmincola to salmonid fish and found that the parasite may cause exophthalmia, intestinal prolapse with anal blockage and damage to fin, tails, gills retina, kidney, pancreas etc. Thus, practically every organ got weakened physiologically. Butler and Millemann (1971) studied the effect of N. salmincola on the swimming ability of juvenile salmonid fishes.

The presence of Ligula intestinalis pleurocercoids has been shown by many workers to be associated with certain pathological effects in fish host (Dogiel et al., 1961). Most of these reports originate in the U.S.S.R. where ligulis present a serious problem in pisciculture (Arme and Wynne-Owen, 1968).

A preliminary study of Ligula infestations by Harris (1973) and Harris and Wheeler (1974) revealed heavy infestation of Ligula in the bleak, Alburnus alburnus, roach and other cyprinids. The data on the infestation indicate an increase in the incidence of infestation in the 3rd year-class bleak, followed by a decrease in older fish and an overall decrease

from summer to autumn. Sweeting (1977) has also described some pathogenic effects of pleurocercoid of L. intestinalis.

A survey of the parasite on fish species of the farm pond in Nigeria (Aderounmu and Aeniyi, 1972) showed that 55% of the fishes were infected with endoparasite cestode but no ectoparasite or any external pathological symptoms were found, and the average condition factor values of Hemichromis fasciatus and Tilapia nilotica ranging between 1.62 and 1.83 were indicative of good conditions.

Parasitic diseases of fishes are usually encountered more often than microbial diseases (Rogers, 1978). Some species that may be considered commensals become pathogenic under certain conditions but most obligate parasites are pathogenic and some, such as the ciliated protozoan Ichthyophthririus, cause great losses. Many helminths, like the digenean grubs, appear to be only slightly or moderately pathogenic, but destroy the aesthetic value of fish.

Eure (1976), who studied the seasonal abundance of Neoechinorhynchus cylindratus (acanthocephalan) in the largemouth bass (Micropterus salmoides) in a heated reservoir, found autumn to be the most intense parasite recruitment season. He considered changes in the fish feeding behaviour and temperature regimes as the principal factors responsible

for the seasonal incidence and intensity pattern of this parasite.

Berra (1978) studied the incidence of Black Spot desease in fishes and observed that 89% of fish were infected with one or more metacercariae of the strigied flukes, Uvulifer ambloplitis Rhinichthys atratulus, Semotilus atromaculatus and Compostoma anomabum had the highest incidence of infection and the greatest number of individual parasites.

A review of literature reveals that Isoparorchis hypselobagri usually infests the swim bladder of siluroid fish (Mahajan, 1978). The degree of infestation in the murrel could be realised from the fact that these parasites weighed 3.13% of the host's body weight.

Mahajan et al. (1979), on the other hand, had reported the effects of this digenean parasite in the pond murrel, Channa punctatus. This parasite was found to induce morphological changes, including partial necrosis of fin tissue, scale loss, considerable damage to the viscera, especially the gonads, and the abdominal muscles.

Rao and Rao (1970) studied the seasonal occurrence of Elongoparorchis pneumatis in the air bladder of the catfish,

Arius thalassinus from the Bay of Bengal. 30 out of 78 fish examined by him during the period January-December, 1969 were found infected with a total of 65 flukes. The parasites were present throughout the year except in May and July when E. pneumatis was reported to be present in the intermediate host.

Jain et al. (1976) made observation on some histopathological changes in the stomach wall of the siluroid, Heteropneuestes fossilis, infected with a cestode. The fish showed loss of appetite, sluggishness and weight loss. At the point of attachment the villi broke down and blood vessels became congested.

Ahmad and Sanaullah (1979) have recorded pathological changes induced by caryophyllid cestode in Clarias batrachus.

Ivanova et al. (1976) reported parasites and diseases in several species of buffalo fish and the channel catfish that have been successfully introduced into ponds in different regions of the U.S.S.R. The introduced species were not found susceptible to infectious diseases commonly occurring in carp. The most commonly occurring parasites were Ichthyophthirius multifilis, Gyrodactylus schulmani, Diplostomum spathaceum and Piscicola geometra.

The ecology, life cycle and host-parasite relationships of Posthodiplostomum minimum (Trematoda : Diplostomatidae have been reviewed and factors influencing the incidence and intensity of infection of metacercariae in fishes reported by Spall and Summerfelt (1970). The incidence of infection of P. minimum was found to vary with age and sex of the fish, with parasitism being greater in males of all the species.

Several authors in the past have reported one or a combination of harmful effects, such as reduced growth, emaciation or smaller average length in heavily parasitized fish when compared with uninfected fish from the same lake. Hunter (1938) observed statistically significant weight loss in smallmouth bass experimentally infected with strigeoid cercariae of Uvulifer ambloplitis. Hugghins (1959) reported that fish with heavy metacercarial infections were 10% shorter than those with lighter infections. Bangham (1938) noted that fish with heavy infections of metacercariae had noticeably less visceral fat and were often thin.

Although the pathogenicity of fish parasites is attracting increasing attention of parasitologists, comparatively little is known of the effects of nematodes on their fish hosts (William, 1967). The study of pathogenic effects of nematode seems one of the most neglected fields in fisheries helminthology.

Intensification of research effort, both in the field as well as in the laboratory, is required to enhance our knowledge of adverse effects of nematodes on their fish hosts. The injurious effects of Larval Anisakidae (Ascaridoidea), particularly in infecting the liver, have however, been thoroughly investigated (Margolis, 1970).

Lee (1981), who studied the ecology in Perca fluviatilis of Acanthocephalus lucii, observed that about 8% of the population was infected with a mean intensity of 2.6% worms per infected fish, the incidence and intensity of infection being greater in older than in the younger specimens.

Acanthocephalus lucidus, an amphibian acanthocephalan, was found in the intestine of the rainbow trout, Salmo gairdneri (Nagasawa and Egusa, 1981). Morphologically this species is characterized by the backward increase in the hook length and the elongated cement gland.

A number of workers have reported Pallisentis ophiocephali as a commonly occurring helminth parasite in different species of murrel of the Indian region (Bhalerao, 1932; Agarwal, 1958; Farooqi, 1958; Saeed and Bilquees, 1971; and Sahai et al.,1971).

George and Madakal (1981) observed the intestinal pathology of the marine fish, Rachycentron canadus, infected

with the acanthocephalid worm, Serrasentis nadakali. Various pathological changes, such as hyperplastic, hypertrophic, destruction of the villi, degeneration and necrosis of the mucosal epithelium, excessive mucus secretion, have also been observed.

Wabuke and Bunoti (1980) studied the prevalence and pathology of the cestode, Polyonchobothrium mossambicus. These were more abundant in the gall bladder and caused pronounced nodular outgrowth in the mucosa. The intensity of infection was greatest in the young fish.

The nature of infection of the liver of Diodon hystrix by the pleurocercoid larvae of Gymnorhynchus gigas has also been observed (Radhakrishnan and Nair, 1981). The parasite appeared to be both host and tissue specific with high incidence of infection in colder months. Fish of intermediate size were more infected than the smaller and larger ones, and males were more susceptible to infection than the females. The histopathological change was in the form of a typical inflammatory reaction aimed at encapsulating the parasite in fibrous connective tissue cyst.

A histological study on the ancylodiscoidosus in the catfish, Silurus glanis, showed that massive infection resulted in fatality within 24 hours due to attachment of

the larvae all over the integument (Molnar, 1980).

The histopathology of acute and chronic infections of rainbow trout, S. gairdneri, with eye flukes, Diplostomum sp. was studied by Shariff et al. (1980), who observed only few clinical signs with acute infection, though in chronic cases the fish became blind, exophthalmic and emaciated.

Sircar and Sinha (1980), who made observations on the histopathology of Lytocestus indicus infection in the fish, Clarias batrachus, noted various histopathological changes, such as hyperplasia, hyper trophy of the villi, increase in the number of connective tissue cells, localised giant cell reaction in the submucosa, etc.

Sommerville (1981) has given a comparative account of the tissue response to invasion and encystment by Stephanochasmus baccatus (Digenea : Acanthocolopidae) in four species of flat fish.

Ocular metacercarial infection of the toadfish, Opsanus tau, was reported by Riis et al. (1981) who observed microscopically digenean metacercariae encapsulated in the periocular tissues, cornea, sclera, occasionally in the iris, causing various pathological changes.

A survey of the prevalence and distribution of proliferative kidney disease (PKD) in England and Wales showed that the disease is a major problem in farmed rainbow trout, S. gairdneri, in several European countries (Bucke et al., 1981). The disease is reported to be seasonal and primarily affecting o^+ fish on river-fed sites in summer and early autumn.

The seasonal variation in the prevalence of Red Spot Disease in estuarine fish, with particular reference to the sea mullet, Mugil cephalus, was recorded by Rodgers and Burke (1981). Disease Epizootics appeared to be related to the occurrence of certain environmental changes,like low oxygen, rapid changes in temperature and rapid or prolonged depressions of salinity in the estuarine habitat. Simultaneously conducted microbiological investigations indicated that the bacterium Vibrio anguillarium was the causative agent of red spot epizootics.

Pickering and Christie (1980) studied the sexual differences in the incidence and severity of ectoparasitic infestation of the brown trout, S. trutta. Sexually mature male fish were more frequently or more severely infested by species of Ichthyophthirius, Scyphidia, Gyrodactylus and Saprolegnia than immature fish of either sex or mature female

fish.

From the above account it is clear that though considerable work has been done on parasitic diseases of both marine as well as fresh water fishes, studies on the incidence of infection of helminth parasites and their pathology, particularly from the north Indian environment, have been rather limited.

An all India Symposium on "The diseases of finfish and shellfish" was organised at the College of Fisheries Mangalore(March 1-3, 1982). This seems to be the first such symposium ever to be held in India. The symposium deliberations assume significance in view of the greater expansion of the culture fisheries in the country, on more intensive scale, and the realization of the fact that proper management of fish health in a pond is a pre-requisite to the economic viability of any aquaculture practice.

The present investigation was initiated with a view to studying the incidence of infection of different helminth parasites, their qualitative and quantitative analysis and the pathological changes caused by them in the common pond murrel, Channa punctatus (Bloch), and the findings are presented by the author in the form of this dissertation.

It is presumed that analysis and establishment of various statistical relationships and histopathological studies would be useful to analysing the harmful effects of different helminth parasites in C. punctatus and the data would be of interest to fishery experts and dieticians.

M A T E R I A L S A N D M E T H O D S

MATERIALS AND METHODS

Live specimens of Channa punctatus were obtained at monthly intervals from the Aligarh fish market and their total length (cm), weight (gm) and sex recorded. In each month at least 20 specimens were dissected out and different parts and organs of their body examined for various helminth parasites.

Collection and fixation of parasites

The parasites from infected tissues were collected in petridish containing 1% normal saline and washed several times with saline in order to remove the debris. The parasites were fixed in 70% alcohol. Active worms were, however, first placed in hot 70% alcohol to achieve complete relaxation of the parasites. The parasites were dehydrated through ascending grades of alcohol, cleared in xylene for about 30 minutes, stained with borax carmine and mounted in canada balsam. The slides were studied under the microscope using different magnifications for identification.

The relative weight of parasite to host tissue has been expressed in terms of parasitization Index (P.I.) adopting the following formula as suggested by Arme and Wynne-Owen (1968)

$$P.I. = \frac{\text{Total weight of parasites per host (g)}}{\text{weight of host plus parasites (g)}} \times 100$$

The age of individual fish was not directly determined, but specimens of C. punctatus were arranged into length estimates corresponding to year-classes $0^+ - 4^+$ as given by Qasim and Bhatt (1966).

The coefficient of condition (K) in normal and infected fish samples was determined, with the objective of expressing the 'condition' or well-being of fish in numerical terms using the general equation

$$K = \frac{W}{L^3} \times 100$$

where, L was the total length of the fish in cm and W was the weight of fish in g.

For studying the pathological effects of parasites on the host tissue, preparations of both the normal and infected organs of fish were made using the conventional micro-techniques

(Swarup et al., 1981).

Fixation and embedding of host tissue

Portions of tissues of both normal and infected fish were fixed in 15% formaline solution. These were then dehydrated through ascending grades of alcohol and cleared in xylene for about 30 minutes. Infiltration of wax was carried out in two changes of paraffin wax (56°C) and the tissues embedded in the paraffin wax.

Section cutting

Sections of the tissues were cut at 7-10 microns on a rotatory microtome. The ribbons of the material were affixed on albumin coated slides and stretched by floating on water drops and exposing to moderate heat of the stretching box. The slides were dried and kept for staining.

Staining Technique

Sections of the slides were transferred to xylol for dewaxing, hydrated through descending grades of alcohol and stained with haematoxyline and eosin. These were then dehydrated through ascending grades of alcohol, cleared in xylene for 15 minutes and mounted in D.P.X.

The prepared slides of both normal and infected host tissue were examined under the microscope to assess the pathological effects of parasites on the host.

Statistical analysis

The correlation coefficient (r) for the various relationships were computed by the following equation.

$$r = \frac{N \times \log xy - \log x \times \log y}{N \times \log x^2 - (\log x)^2 - (\log y)^2}$$

The statistical significance of the coefficient conditions (K) was calculated through t-test.

Methods for further statistical evaluation of the data were the same as given by Snedecor (1959).

C H A P T E R - I

INCIDENCE OF INFECTION OF HELMINTH PARASITES AND THEIR EFFECT ON THE 'CONDITION' OF THE FRESHWATER POND MURREL, *CHANNA PUNCTATUS* (BLOCH)

INTRODUCTION

Among the important problems encountered in modern fish culture are the parasites and diseases. A majority of fishes carry heavy infection of parasites which could cause deterioration in their food value and may even result in their mortality.

As has been discussed earlier (Page 3), in India, although much attention has been paid towards the study of helminthiasis in various farm animals, information on this aspect in fish, particularly from the fresh water environment, is relatively limited.

The present account deals with the incidence of infection of different helminth parasites and their effect on the 'condition' of the common pond murrel, *Channa punctatus*, leading to the establishment of some interesting statistical relationships. The findings are considered significant in

view of the current interest in murrel culture in this country.

MATERIALS AND METHODS

Details of the methods of fish sampling, determination of the age, coefficient of condition of the fish and parasitization index, and methods of fixation and collection of parasites have been described elsewhere (see page 17-20).

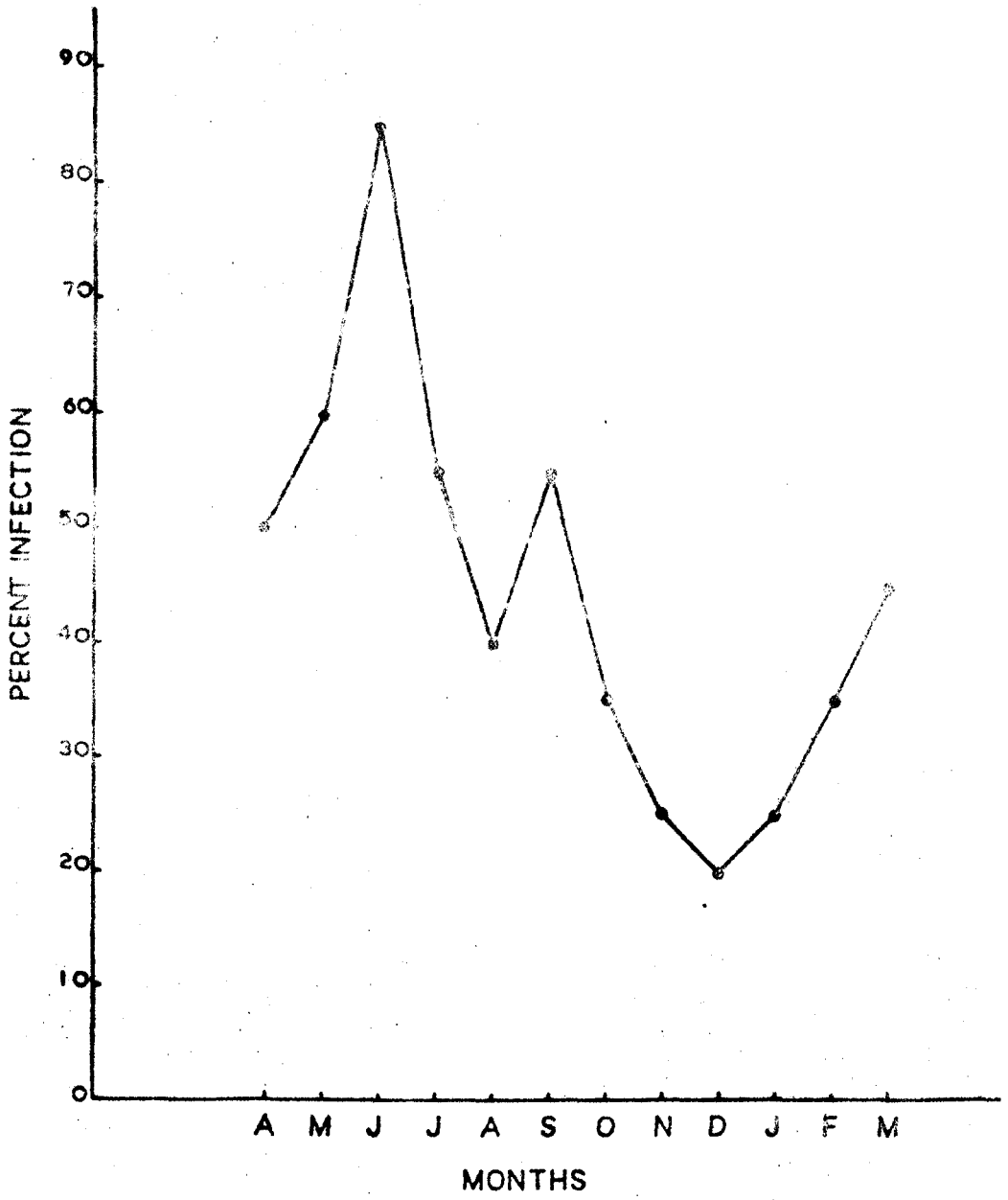
RESULTS AND DISCUSSION

Three different species of helminth parasites, namely, Clinostomum complanatum, a trematode, Ascarops nema, a nematode, and Pallisentis ophiocephali, an acanthocephalan, were recorded in the murrel, Channa punctatus.

The acanthocephalan (P. ophiocephali) were mostly found located in the intestine of the host. C. complanatum were found attached to the liver and kidney, while A. nema occurred in the body cavity of the host.

The number of host specimens examined to those found infested, and the numerical strength of the helminth species recorded, formed the basis for a quantitative evaluation of

Fig. 1 Mean incidence of infection of helminth parasites in Channa punctatus

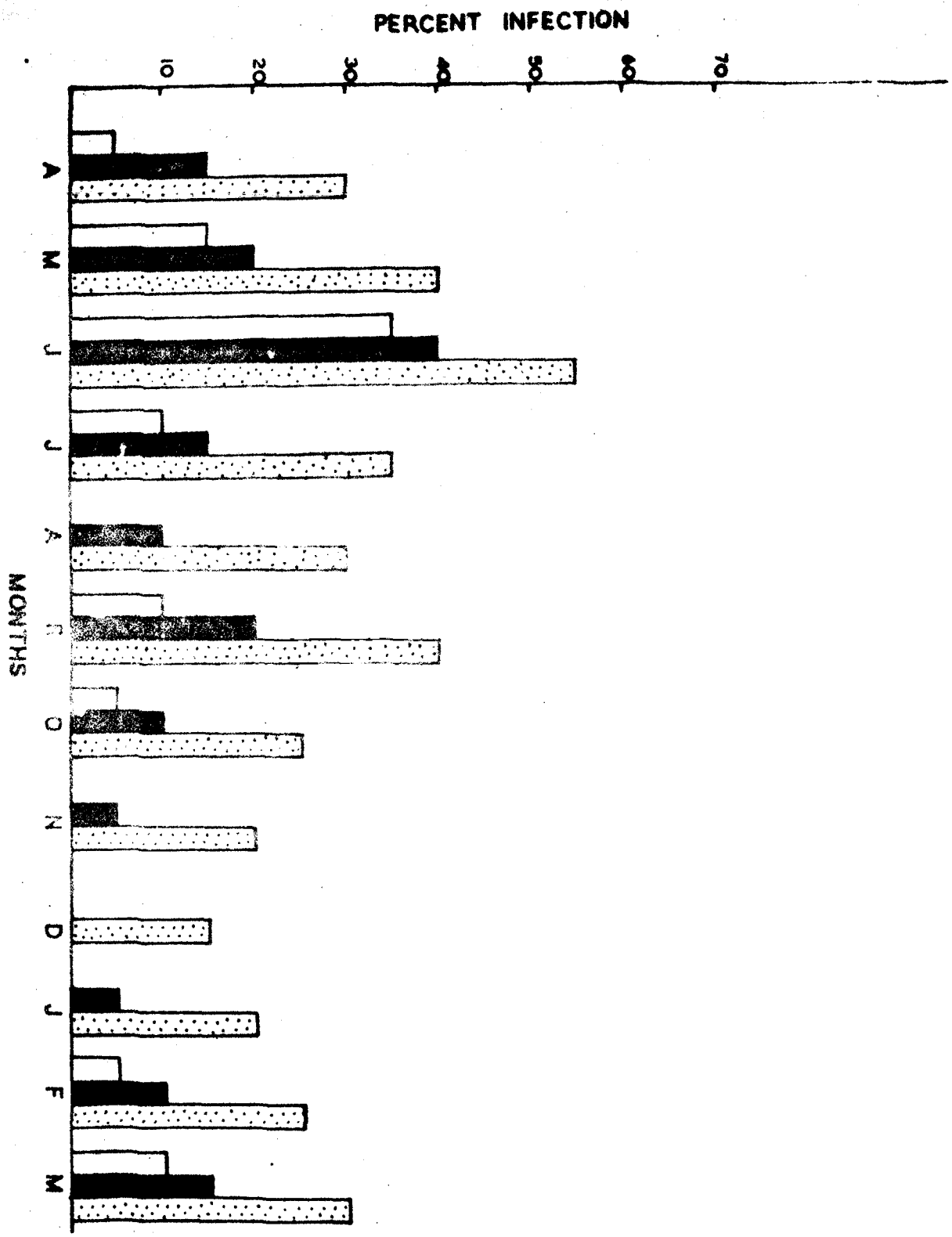

PERCENT INFECTION
90
80
70
60
50
40
30
20
10
0
A M J J A S O N D J F M
MONTHS

the data. Out of the total number of 240 specimens of Channa punctatus examined, a total of 107 individuals were found infected with a total of 316 helminths belonging to the above mentioned species. The total percentage of infected host was around 45%. There seemed to exist a considerable degree of individual variation in the intensity of infestation of different helminth parasites. The intensity of invasion per individual host ranged from 1-6 for P. ophiocephali, 1-11 for C. complanatum, and 1-15 for A. nema. The percentage of mean number of helminth parasites per infected host also varied with different helminth species. It was found to be 2.13% for P. ophiocephali, 2.3% for C. complanatum and 4% for A. nema. Thus, the mean intensity of infection of A. nema was higher than that of C. complanatum or P. ophiocephali (Table I).

The incidence and abundance of helminth parasites in C. punctatus exhibited an interesting pattern. The level of incidence of infestation for P. ophiocephali was higher (13.2%) than that for C. complanatum and A. nema, where the values were observed to be 13.3% and 8.3%, respectively (Table I).

As could be seen from Table II, 41 males and 66 females were found to harbour the helminth parasites and the infection

Fig. 2 Incidence of infection of different helminth parasites in *Channa punctatus* (□ - *Ascarops nema*; ■ -*clinostomum complanatum*; ▯ -*Pallisentis ophiocephali*)


PERCENT INFECTION
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MONTHS
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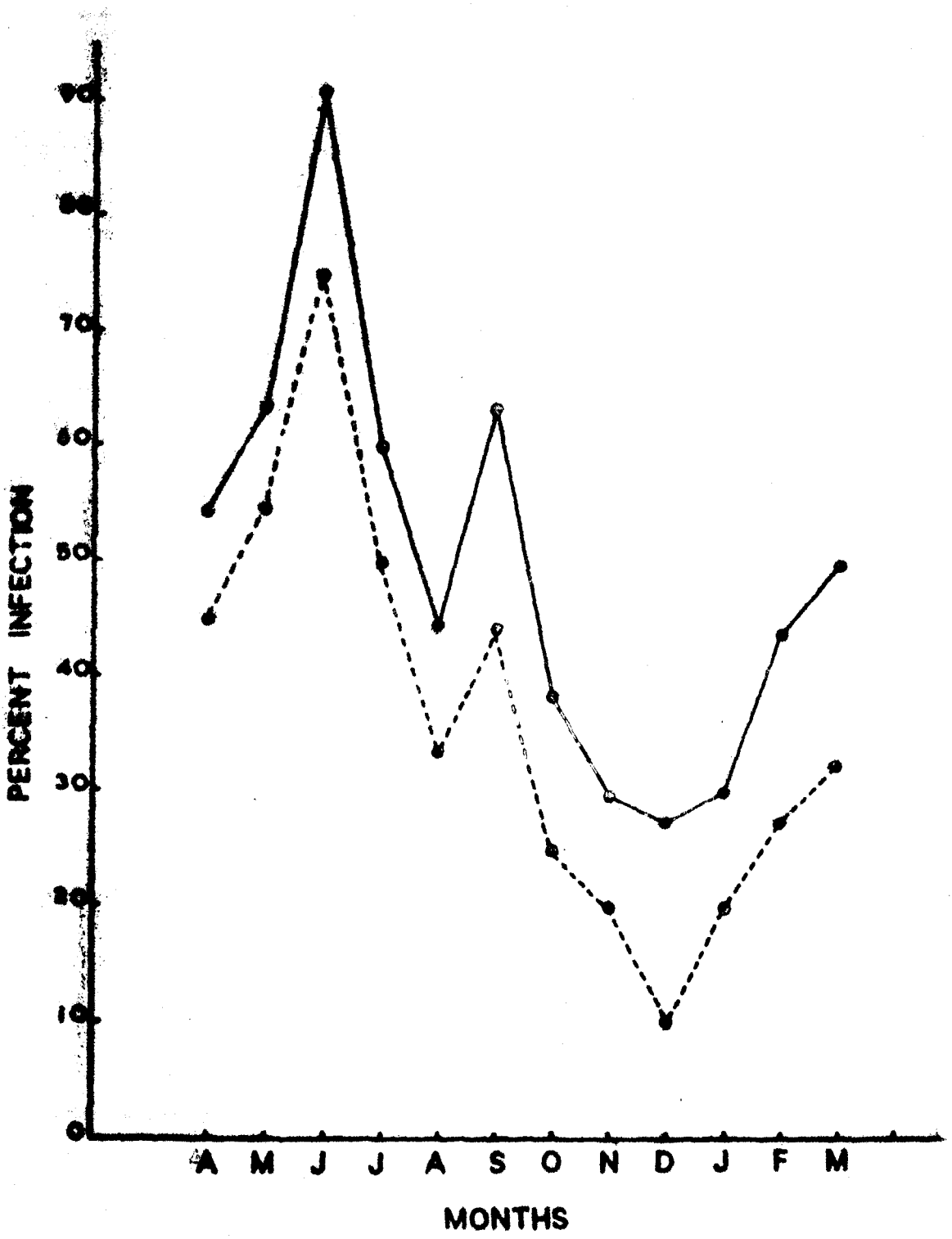
M

was present almost throughout the year. However, the degree of incidence of infection of each of the above three helminth parasites showed marked variations with seasons. The incidence of infection for all the three helminth species was high during the summer season with a peak (85%) in the month of June and a trough (20%) in the month of December (Fig. 1). Species-wise (Fig. 2), the incidence of infection was highest for P. ophiocephali and it existed throughout the year. For C. complanatum, however, the incidence of infection was almost zero in the month of December. Similarly, a zero level of incidence of infection was observed for A. nama in the months of August, and November to January.

The seasonal variation in the rate of helminth infection may be related to the temperature of the environment. The rise in temperature during summer resulted in an overall increase in the rate of parasitic infection, while a decline in temperature during winter showed a decrease in the rate of parasitic infection. Johansson (1978) has made a similar observations on the Swedish salmon.

An analysis of the ratio of worm burden/fish in the two sexes have also been computed. It revealed that the female fish harboured more parasites than the males. Average worm burden/fish was found to be 103 in males and 213 in

Fig. 3 Incidence of infection of helminth parasites in the two sexes of Channa punctatus. (——— , Male; —— , Female)


PERCENT INFECTION
70
60
50
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A
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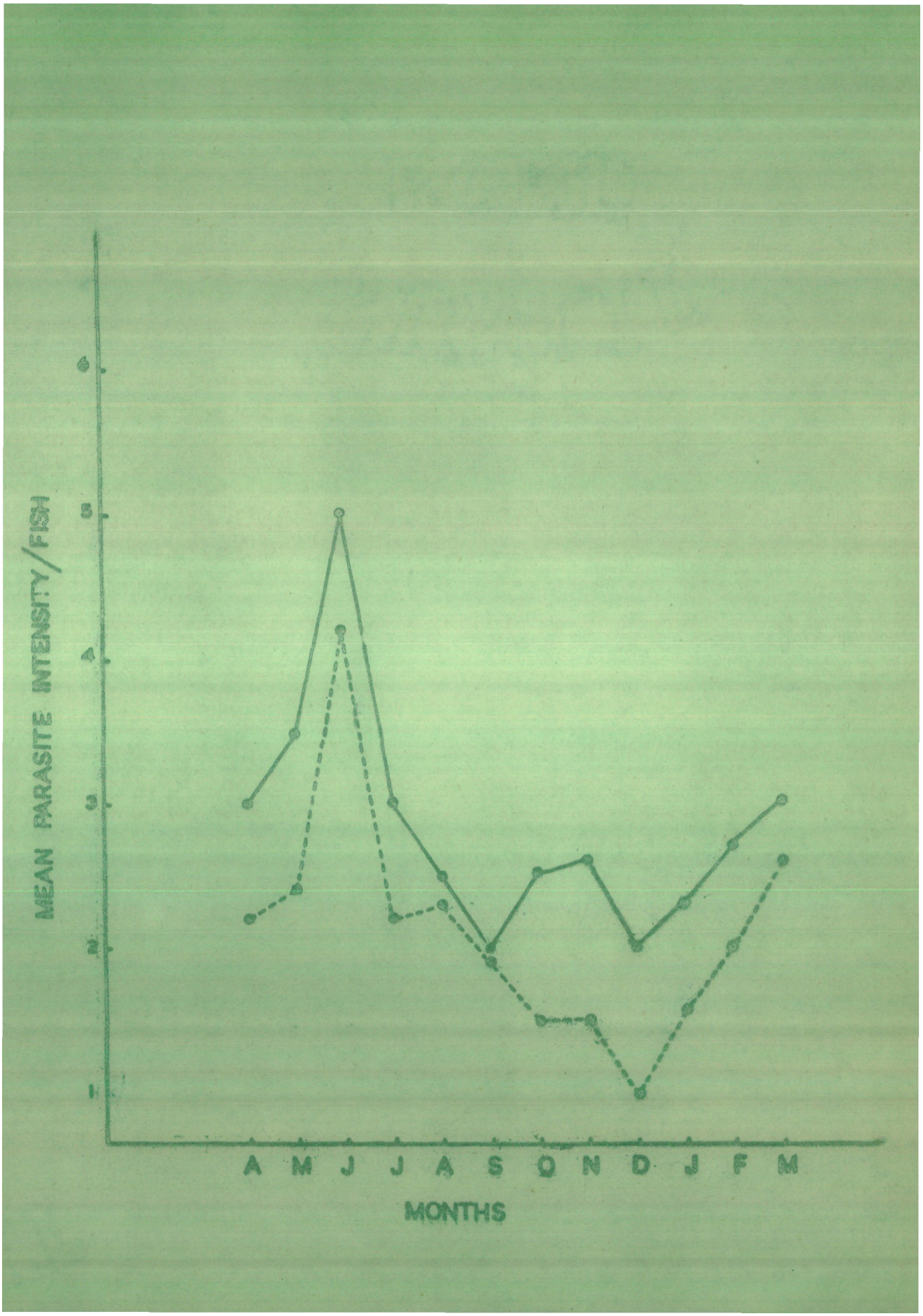
MONTHS

females with a ratio of 1:2. The data pertaining to the sex-related difference in the worm burden of fish have been presented in Table II. The infection in terms of percentage during the total period of investigation was 50% in female as against 33% in male hosts (Table II, Fig. 3). This difference in the extent of helminth infection in the two sexes of the host could be related to some known facts in the biology and/or physiology of C. punctatus. The female fish, subjected to greater physiological stress as a result of gonad maturation during the pre-spawning months (April-June), becomes more susceptible to infection, and it is during this period that the fish were observed getting heavily infected with different helminth parasites. It may be pointed out that the fish is known to indulge in more active feeding during this period (Qayyum and Qasim, 1964). A fall in the rate of feeding during the peak spawning months (July-August), however, coincides with a decline in the rate of parasitic infection in both sexes. Kennedy (1975), while studying the level of infection in the fresh water fishes, has pointed to a similar spawning stress and diet effect in the female hosts. Bure (1976) has considered changes in the fish feeding behaviour and annual temperature regime as the principal factors responsible for the seasonal

Fig. 4 Seasonal Variations in the intensity of helminth infection in *Channa punctatus* (—— , Male; —— , Female)


MEAN PARASITE INTENSITY/FISH
1
2
3
4
5
6
A M J J A S O N D J F M
MONTHS

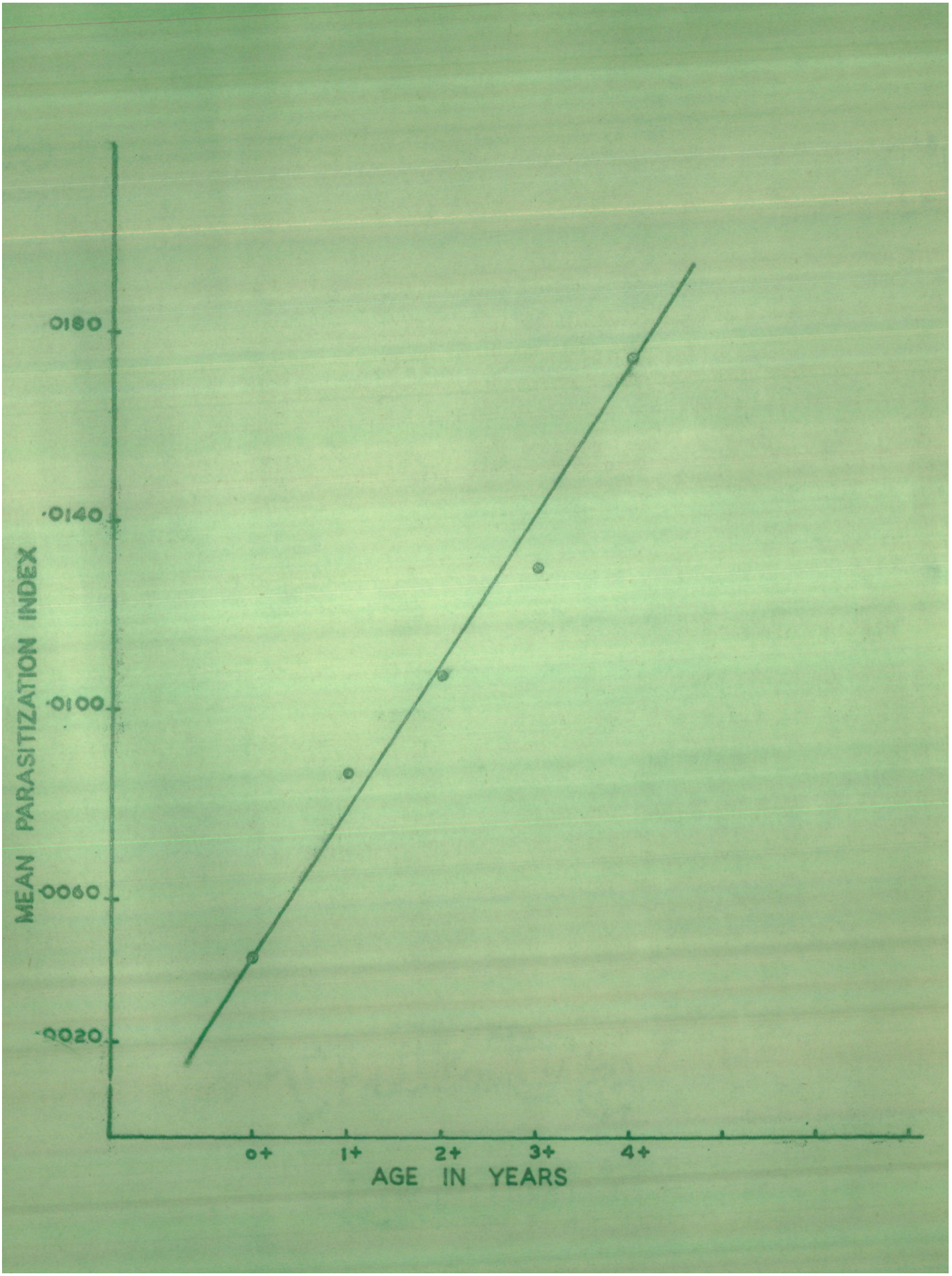
incidence and intensity patterns of parasites.

Evidence on the increase both in the incidence and abundance of some parasites with the onset of maturity in the fish hosts is also available in the work of Chem and Power (1972) and Hare and Burt (1975). It is also possible that the female fish may be possessing a positive stimulus which preferentially attracts the helminth parasites. Conversely, the male fish may be having a stronger in-built resistance to the infection leading to the establishment of fewer parasites in them as has been pointed out by Siddiqui and Nizami (1982).

It is interesting to note that the difference in the intensity of infection of the two sexes of the murrel was almost a year round phenomenon, except in the month of August and September when the level of infection was more or less identical in both the sexes (Fig. 4).

In C. punctatus, infection by a single helminth species predominated making up 89% of all infections. There were nine (8.4%) cases in which a host was found infected with two helminth species and one to two (1.8%) cases in which infection was confined to three helminth species. The multiple infection with two or more of the helminth species were more frequent (9%) in females than in males where it was only about 2%.

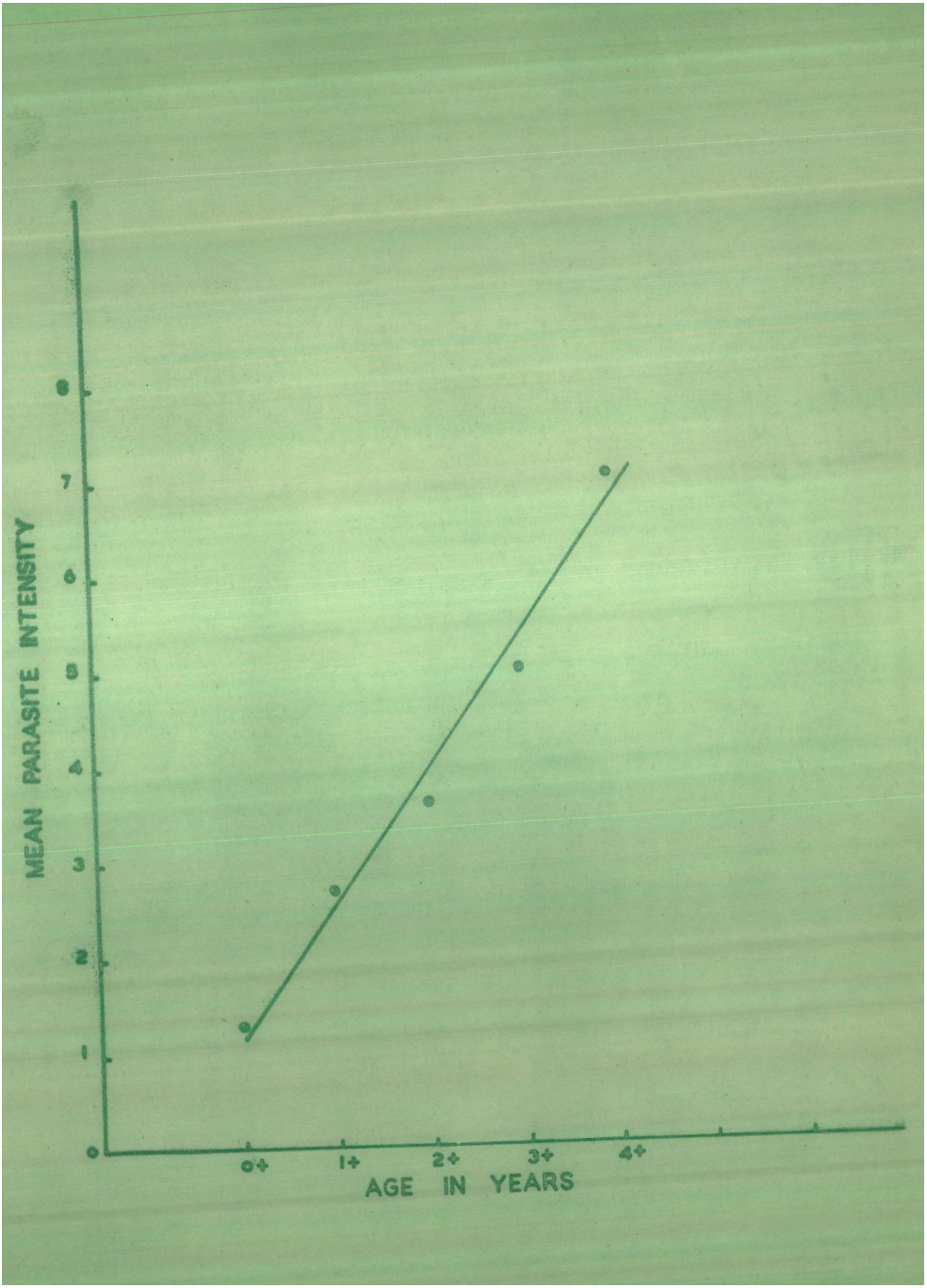
Fig. 5 Relationship between the mean parasitization index values in different age-groups of *Channa punctatus*.


MEAN PARASITIZATION INDEX
·0180
·0140
·0100
·0060
·0020
0+
1+
2+
3+
4+
AGE IN YEARS

The values of mean parasitization index (M.P.I.) increased with the age of the fish (Table III). The mean parasitization index was lower (0.0038) in younger (0^+) year-class, but higher (0.0176) in older (4^+) year-class fish (Table III). The values plotted in Fig. 5 for the mean parasitization index of C. punctatus belonging to different age-groups indicate that the worm load increases linearly with the age of the host. Similarly, the incidence of infection of different helminth parasites, when computed in relation to age of the fish, showed a higher (65%) incidence of infection in older (4^+) year-class than in younger (0^+) year-class fish where it amounted only to 36.5% (Table III). Furtado (1973) has pointed out that in the freshwater catfish, Calrias batrachus, the incidence of infection is related to the size with the larger hosts having a higher infestation rate.

The mean parasite intensity also showed a linear relationship with the age of the host (Table III, Fig. 6). On an average, the numerical strength of helminth parasites was 1.3 in younger fish belonging to 0^+ year-class and 7.3 in individuals of the higher 4^+ year-class. The direct correlation between parasite intensity and the age of the host could be attributed to increase in the living space with the growth of the fish.

Fig. 6 Relationship between the mean parasite intensity in different age-group of *Channa punctatus*.


MEAN PARASITE INTENSITY
0
1
2
3
4
5
6
7
0+
1+
2+
3+
4+
AGE IN YEARS

The relationship between the weight of parasite and fish length has also been worked out statistically and expressed by the following regression equation.

$$\text{Log } L = 0.955 + 1.31 \text{ log } W.$$

where Log L, was the length of the infected fish in mm; and log W was the weight of the parasite in mg.

The correlation coefficient ($r = 0.955$) for this relationship was found to be significant ($P < 0.001$). This direct relationship between length of the infected fish and weight of the parasite indicates that the worm load or total weight of the parasite also increases with the fish size. The positive relationship between the weight of the parasite and the length of the host has also been discussed by Harris and Wheeler (1974).

The coefficient of condition (K) have been widely used by fishery investigators to express the relative robustness of fishes. It has also been used in age and growth studies to indicate the suitability of an environment to a fish species (Lagler, 1982).

A few investigators have either compared the condition factor (K) of the parasitized and non-parasitized fish, or computed the correlation between the condition factor and

number of parasites (Elliot and Russert, 1949; Fox, 1962; Rabideau and Self, 1953; and Lewis and Nickum, 1964).

During the present investigation the coefficient of condition value have been computed for all the specimens of C. punctatus in an attempt to make an assessment of effect of parasitization on the 'condition' of the fish.

The average values of coefficient of condition recorded for the normal and infected fish were found to be 1.61 and 1.20, respectively. Differences between the 'K' values obtained for the normal and infected fish, as assessed with t-test, were found to be significant ($P < 0.001$), indicating that the helminth parasites have affected the 'condition' of their fish host.

A comparison has also been made between K-values of infected specimens of C. punctatus belonging to different size-groups (Table IV). It is evident from the data that, although the condition of fish of all size group was affected by parasitic infestation, the fish of larger size-group, though possessing a greater worm load, seemed less adversely affected than the smaller ones.

Studies on the relationship between length and weight of fishes constitute an important aspect of their biology.

Besides providing a mathematical relationship between length and weight of fish as a mean of interconversion, such a relationship is considered important in the study of population dynamics, and also yielding information on aspects, like the general well-being of the fish, relative robustness, growth variation, size at first maturity, gonad development and breeding season, etc. (Chatterji et al., 1977; Sultan and Khan, 1981; Lagler, 1982).

In order to evaluate the effect of parasites on the length-weight relationship of C.punctatus, this relationship was calculated both in the normal as well as in the infected individuals, and expressed by the following regression equation.

$$\text{Log } L_1 = 0.49 + 1.709 \text{ Log } W_1 \quad \ldots\ldots \text{(i)}$$

$$\text{Log } L_2 = 0.27 + 1.602 \text{ Log } W_2 \quad \ldots\ldots \text{(ii)}$$

where L_1 and L_2 were the total body lengths (cm) of the normal and infected fish, W_1 and W_2 were the weights (g) of the normal and infected fish, respectively.

The correlation coefficient (r) for the above relationship were found to be 0.940 for the normal ($P < 0.001$) and 0.460 for the infected ($P < 0.1$) fish.

It is evident from the data that the length weight relationship was more strong and significant in the normal fish than in the infected ones, indicating that the parasites have disturbed the normal growth pattern of the fish.

SUMMARY

Three different species of helminth parasites, namely, C. complanatum, P. ophiocephali and A. nema, were recorded in the freshwater pond murrel, C. punctatus and these showed significant seasonal variations. The rate of parasitic infection was found related to some such factors, like temperature, rate of feeding and maturation cycle of the fish. The level of infection was higher during the warmer months, attaining a peak (85%) in the month of June. Infection by a single helminth species (P. ophiocephali) predominated, making up about 89% of all the infections. The cases of multiple infection with two or more of the helminth species were more frequent in females than in males. In terms of mean parasite intensity, higher value was recorded for A. nema than for P. ophiocephali or C. complanatum.

Average worm burden/fish was found to be 103 in males and 213 in females with a ratio of 1:2. During the year of study, 50% female and 33% male fish were found to be infected

with one peak in June.

The value of the mean parsitization index increased with the age of the fish host. The 'condition' of the fish, as established by the coefficient of condition (K), as also the length-weight relationship, were found affected by the harbouring parasites.

TABLE - I : Data showing different helminth parasites infection in *Channa punctatus*.

Helminth Species	Number of infected Fish	Percent Host Infected	Total number of helminth (%)	Mean intensity
Acanthocephala *Pallisentis ophiocephali*	75	31.2%	160 (46.8%)	2.13
Trematode *Clinostomum complanatum*	32	13.3%	76(42.1%)	2.3
Nematode *Ascarops sp.*	20	8.3%	80(25%)	4
Total	107	45%	316(33%)	2.5

TABLE - II : Seasonal Variation in the incidence and intensity of helminth infection in *Channa punctatus*

MONTHS	Number of individual examined			Number of individual infected by			Total number of fish infected			Total worm Burden		Helminth parasites ratio	
	♂	♀	Total	*Ophiocephali*	*C. Complanatum*	*Anema*	♂	♀	Total	♂	♀	♂	♀
April	9	11	20	6	3	1	4	6	10	9	18	1	20
May	9	11	20	8	4	3	5	7	12	12	25	1	20
June	8	12	20	11	7	8	6	11	17	28	56	1	20
July	10	10	20	7	3	2	5	6	11	11	20	1	18
August	9	11	20	6	2	-	3	5	8	7	15	1	21
September	9	11	20	8	4	2	4	7	11	7	14	1	20
October	8	12	20	5	2	1	2	5	7	3	10	1	31
November	10	10	20	4	1	-	2	3	5	3	8	1	26
December	9	11	20	3	-	-	1	3	4	1	6	1	20
January	11	9	20	4	1	-	3	3	6	5	7	1	35
February	11	9	20	5	2	1	3	4	7	7	15	1	21
March	8	12	20	7	3	2	3	6	9	8	18	1	22

TABLE - III : The level and intensity of helminth infection in *Channa punctatus* belonging to different age-groups.

Age Group	Number Examined	Percent Infection	Mean parasitization Index (M.P.I.)	Mean Intensity
0^+	68	36.5%	.0038	1.3
1^+	75	40%	.0087	2.7
2^+	15	60%	.0108	3.6
3^+	26	63%	.0131	4.6
4^+	20	65%	.0176	7.3

Table - IV : Mean values of Coefficient of condition in the normal and infected Channa punctatus belonging to different size-groups

Size Group	Mean Coefficient of condition ('K') of normal Fish	Mean Coefficient of condition ('K') of infected Fish
0^+	1.54	1.10
1^+	1.58	1.16
2^+	1.62	1.20
3^+	1.65	1.25
4^+	1.68	1.30

C H A P T E R - II

HISTOPATHOLOGICAL OBSERVATIONS ON THE TISSUES OF THE POND MURREL, *CHANNA PUNCTATUS* (BLOCH), INFECTED WITH HELMINTH PARASITES

INTRODUCTION

Studies in the past have indicated that the helminth parasites in fish tissues may cause considerable pathological changes. A number of workers have studied the histopathological changes caused by acanthocephalan parasites. Prakash and Adams (1960) have studied the histopathology of intestinal lesions induced by *Echinorhynchus lageniformis*. Bullock (1961) has studied the histopathological effects of *Acanthocephalus jacksoni* in certain salmonid fishes of North America. Similarly, Hasan and Yasim (1960) have reported the destruction caused by *P. basiri* in the liver of *Trichogaster chuna*. Schmidt *et al.* (1974) have made some histopathological observations caused by acanthocephalan infection in the vertebrate intestine. Rydlo (1975) has reported the pathogenicity and transmission possibilities of fish acanthocephalans.

Pathological significance of the metacercariae of Clinostomum has been recorded by Rai (1968) in the three species of pond fishes, namely, C. punctatus, C. batrachus and H. fossilis.

Histopathological changes caused by different helminth parasites in the murrel, C. punctatus, examined during the present investigation have been briefly described in this chapter.

MATERIALS AND METHODS

Techniques of the preparation of slides of normal and infected organs have been described earlier under 'Materials and Methods' (page 17- 20)

RESULTS AND DISCUSSION

Sections of the intestine and liver of C. punctatus infected respectively with P. ophiocephali and C. complanatum were examined and histopathological changes recorded.

The main histopathological changes observed to be caused by P. ophiocephali in the intestine of its fish host was local

intestinal damage. The worm attached to the intestinal wall caused destruction of the villi, degeneration and necrosis of the mucosal epithelium (Plate II). Excessive mucus secretion, appearing to be the result of consistant irritation caused by the penetration of the worm species in the submucosa, has also been noted.

The worms were found lengthwise embedded in a copious mucous coat and the intestine at the site of location of worms appeared almost transparent, so much so that the worms could even be seen with the naked eye. Younger forms were generally found with embedded proboscis whereas larger ones occurred lying sluggishly more posteriorly in the embedded intestinal region.

Prolonged infection in C. punctatus with P. ophiocephali also seemed to have adverse effect on the adjoining intestinal tissue (Plate III). In instances of older and heavier infections, the adjoining submucosa of the intestine was found to undergo marked atrophy and necrosis. The villi and the submucosa had probably been secondarily lost.

Some similar histopathological observations in the fish intestine have also been reported by Bullock (1961); Prakash and Adams (1960); George and Nadakal (1981); and

Schmidt *et al*. (1974). The studies show that infestation of acanthocephala in fish intestine result in marked histopathological consequences and these are probably more prominent in fishes than in other groups of vertebrates.

George (1981), while studying the intestinal pathology of the marine fish infected with the acanthocephalid worm, indicated that the worm infection causes hyperplastic and metaplastic as well as hypertrophic changes involving respectively, the connective tissue, epithelial and muscle cells of the fish intestine. Cell type resembling epitheliods, lymphocytes macrophages and cells of unknown identity were reported to aggregate at the infected area in response to inflammation. It has also been postulated that heavy infection might result in interference with intestinal metabolism (Bullock, 1961).

Besides *P. ophiocephali*, *C. complanatum* too are of great pathological importance. Metacercariae of *C. complanatum* were mostly found in the liver tissue of *C. punctatus* in the encysted form. These metacercariae were yellowish in color, sometimes revealing a black pigmentation towards the periphery. The comparatively thicker cyst wall consisted of many layers of reticular fibres with scattered black pigment spots. At

times, two or three juveniles were found lying coiled up inside a cyst.

Infected liver of the fish, when compared with the normal one, showed marked histopathological changes (Plate IV-VII). Liver tissue exhibited pressure atrophy and degeneration of the hepatic cells. Metacercarial cyst also seemed to result in lysis at places of attachment in the liver cell and, subsequently, in the formation of a cavity around the parasite due to the degeneration of hepatic cells. Similar observation has been made by Rai (1968) in the three species of pond fishes, viz. C. punctatus, C. batrachus and H. fossilis.

It is thus evident from the above studies that both P. ophiocephali and C. complanatum are of significant pathological importance and the resulting pathological changes, besides affecting the growth and 'condition' of the murrel, could possibly deteriorate the nutritive value of this important food fish.

SUMMARY

The intestine and liver of C. punctatus found infected by the acanthocephalan parasite, P. ophiocephali and the metacercariae of C. complanatum, respectively, showed significant histopathological changes. The changes caused by P. ophiocephali were mainly destruction of the villi, degeneration and necrosis of the mucosal epithelium and excessive mucus secretion, etc. The pathological changes caused by C. complanatum in the liver were degeneration of the hepatic cells lysis, at the place of attachment and formation of a cavity around the parasite.

Plate I Photomicrograph of T.S. of normal intestine of *Channa punctatus*. (x 50)

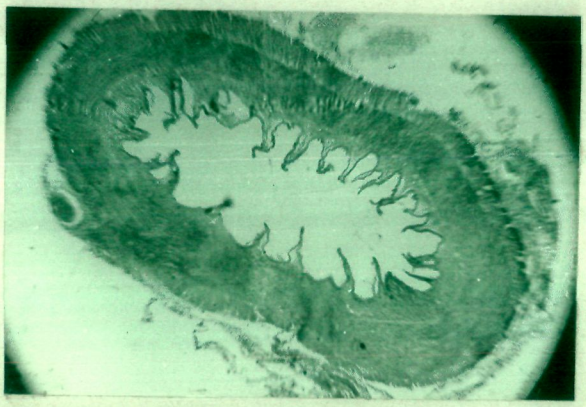


Plate. II Photomicrograph of T.S. of intestine of *Channa punctatus* infected with *Pallisentis ophiocephali* (x 50)

Plate. III Photomicrograph of T.S. of intestine of Channa punctatus infected with Pallisentis ophiocephali (x 100)

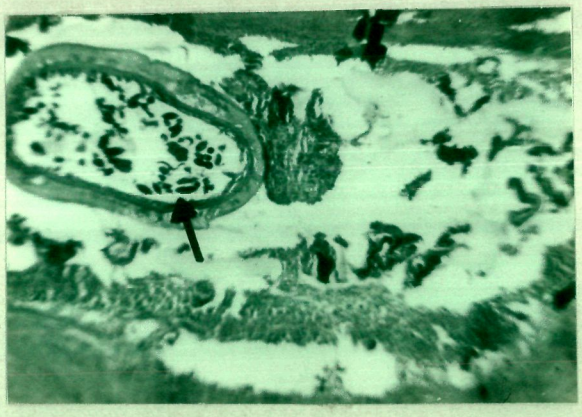


Plate. IV Photomicrograph of a portion of section of liver of *Channa punctatus* infected with *Clinostomum* metacercariae (x 50)

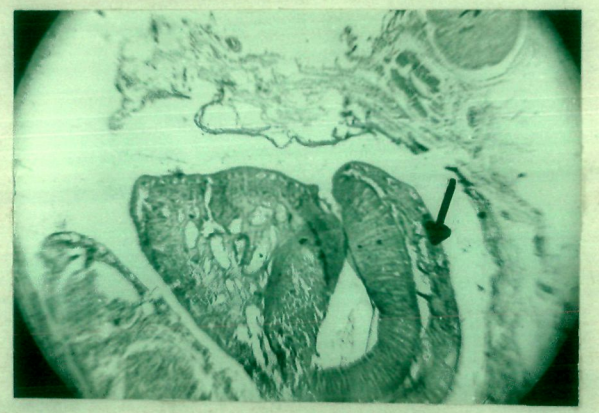
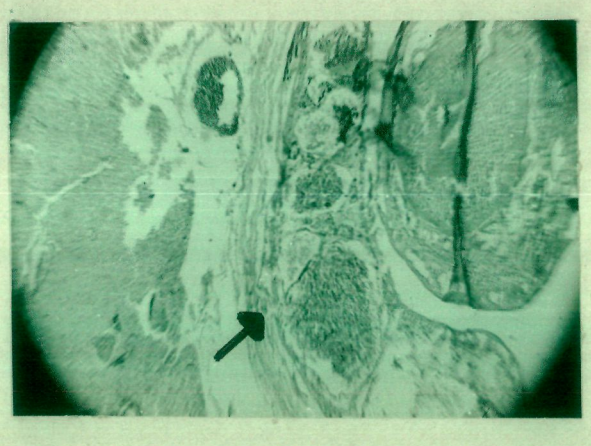


Plate. V Photomicrograph of a portion of section of liver of *Channa punctatus* infected with *Clinostomum* metacercariae (x 50)

Plate. VI Photomicrograph of a portion of section of liver of Channa punctatus infected with Clinostomum metacercariae (x 50)

Plate. VII Photomicrograph of a portion of section of liver of *Channa punctatus* infected with *Clinostomum* metacercariae (x 50)



B I B L I O G R A P H Y

BIBLIOGRAPHY

Aderounmu, E.A. and Aeniyi, F.	1972	Cestodes in fish from a pond at the Ife, Nigeria. Trop. Hydrobiol. Fish. 2(2):151-156.
Agarwal, S.C.	1958	A new Sp of the genus Pallisentis (Acanthocephala) Curr. Sci. 27(3):107.
Ahmad, A.T.A. and Sanaullah, M.	1979	Pathological observations of the intestinal lesions induced by caryophyllid cestodes in Clarias batrachus (Linnaeus) (Siluriformes: clariidae). Fish. Pathol. 14(1):1-7
Amlacher, E.	1970	"Text book of Fish Diseases" (translated by D.A. Conroy and R.L. Herman), TFH Publications, New Jersey.
Arme, C. and Wynne-owen, R.	1968	Occurrence and pathology of ligula intestinalis infections in British fishes. J. Parasit. 54(2):272-280.
Bangham, R.V.	1938	Parasites of centrarchidae from Southern Florida. Trans. Amer. Fish. Soc. 68:263-268.
Bennington, E.	1951	The life history of the salmon poisoning fluke Troglotrema salmincola (Chapin). Ph.D. Thesis, Oregon State Univ., Corvallis, 50 pp.
Baldwin, N.L., Millemann, R.E. and Knapp, S.E.	1967	"Salmon poisoning" disease.III. Effect of experimental Nanophyetus salmincola infection on the fish host. J. Parasit. 53:556-564

Bennington, B. and Pratt, I.	1960	The life history of the salmon-poisoning fluke, *Nanophyetus salmincola* (Chapin). J. Parasit. 46:91-100
Berra, T.M.	1978	Incidence of black spot diseases in fishes in cedar Fork Creek Ohio. Ohio. J. Sci. 78(6):318-322
Bhalerao, G.D.	1932	On a new Sp of Acanthocephala from ophiocephalus striatus. Ann. Mag. Nat. Hist. 7(10):569-573.
Bullock, W.L.	1961	A preliminary study of the histo-pathology of Acanthocephala in the vertebrate intestine. J. Parasit. 47:(4, Sect.2).
Butler, J.A. and Millemann, R.E.	1971	Effect of the salmon poisoning trematode, N. salmincola, on the swimming ability of juvenile salmonid fishes. J. Parasit. 57(4):860-865.
Bucke, D., Seagrave, C.P., Hudson, E.B. and Gregor D.Mc.	1981	A survey of the prevalence and distribution of proliferative kidney Disease (PKD) in England and wales. J. Fish. Dis. 4(5):437-439
Chen, M. and Power, G.	1972	Infection of American smelt in lake Ontario and lake Erie with the microsporidian parasite Glugea hertwigi (Weissenberg). Can. J. Zool. 50(9):1183-1188.
Chatterji, A. Siddiqui, A.Q. and Khan, A.A.	1977	Length-weight relationship of a Carp, Labeo bata (Ham). Proc. Indian Acad. Sci., 86(3):189-194.

Davis, H.S.	1958	Culture and Diseases of Game Fishes. 2nd ed., University of California Press, Berkeley and Los Angles 332pp.
Dogiel, V.A., Petrushevsky, G.K. and Polyanski, yu. I.	1961	Parasitology of Fishes. English translation by Z. Kobata, Oliver and Boya, Edinburgh and London. 384 pp.
Elliot, A.M. and Russert, L.R.	1949	Some condition characteristics of a yellow perch population heavily parasitized by Clinostomum marginatum. J. Parasit. 35(1):183-190.
Eure, H.	1976	Seasonal abundance of Neochinorhynchus cylindratus taken from largemonth bass (Micropterus, salmoides) in a heated reservoir. Parasitology 73(3):355-370.
Farooqi, H.U.	1958	A new Sp of the genus Pallisentis (Acanthocephala) from a fresh water eel. Z. Parasitenk. 18:457-464.
Farrell, R.K. and Lloyd, M.A.	1962	The life cycle of the salmon poisoning fluke. In:Science in Alaska. Proc. 12th Alaskan Sci. Conf., 104-107.
Fox, A.C.	1962	Parasite incidence in relation to size and condition of trout from two Montana Lakes. Trans. Amer. Microsc. Soc. 81:179-184.
Fraser, P.G.	1960	The occurrence of Diphyllabothrium in trout with special reference to an outbreak in the west of England. J. Helminth. 34(½):59-72.

Furtado, J.I.	1973	Incidence of some helminth parasites in the Malaysian Catfish, Clarias batrachus (Linnaeus). Theor. Angew. Limnol. 18(3):1674-1685.
George, P.V. and Nadakal, A.M.	1981	Observation on the intestinal pathology of the marine fish, Raghycentron canadus (Gunther) infected with the acanthocephalid worm, Serrasentis nadakali. Hydrobiologia, 78(1):59-62.
Hare, G.M. and Burt, M.D.B.	1975	Abundance and population dynamics of parasites infecting Atlantic Salmon (Salmo salar) in Trout Brook, New Brunswick, Canada. J. Fish Res. Bd. Can. 32(11):2069-2074.
Harris, J.R. and Hickey, M.D.	1944	Definitive hosts of a species of Diphyllobothrium causing mass infection of trout in reservoirs. J. Path. Bact. 56:310.
Harris, M.T.	1973	A preliminary study of ligula infestations of Bleak in the Thames. Parasitology 67(2):7
Harris, M.T. and Wheeler, A.	1974	Ligula infestation of bleak Alburnus alburnus in the tidal Thames. J. Fish Biol. 6(2):181-188.
Hasan, R. and Qasim, S.Z.	1960	Occurrence of Pallisentis hasiri (Acanthocephala) in the liver of Trichogaster Chuna (Ham). Z. Parasitenk 20:151-156.
Hoffman, G.L. and Dunbar, C.E.	1961	Mortality of eastern brook trout caused by plerocercoids (Cestoda: Pseudophyllidea Diphyllobothriidae) in the heart and viscera. J. Parasit. 47(3):399-400.

Hugghins, E.J. 1959 Parasites of fishes in South Dakota. S. Dak. Exper. Sta. Bull. 484:73pp.

Hunter, G.W. III, and Hunter, W.S. 1938 Studies on host reactions to larval parasites 1. The effect on weight. J. Parasit. 24:477-481.

Ivanova, N.S., Mirzoeva, L.M. and Poddubnaya, A.V. 1976 Parasites and diseases of introduced Sp. Rybn. Khoz.(Mosk.), No.10, 16-17.

Jain, S.P., Pandey, K.C. and Pandey, A.K. 1976 Some histopathological observations on the stomach wall of H. fossilis infected with a cestode. J. Res. Sc. 25(3):1-3.

Johansson, N. 1978 Seasonal Variations related to water temperature in the occurrence of some bacterial and protozoan infections in Swedish Salmon stations. Salm. Res. Inst. Rep., No.3, 8pp.

Kennedy, C.R. 1975 Ecological Animal Parasitology, Oxford : Blackwell Scientific Publication, 67pp.

Lagler, K.F. 1982 Freshwater Fishery Biology. 2nd. ed., Dubuque, W.M.C. Brown Co., Pub., C 1956 XII, 421pp.

Lee, R.L. 1981 Ecology of Acanthocephalus Lucii in Perch, Perca fluviatilis, in the Serpenter London, U.K. J. Helminth. 55(2):149.

Lewis, W.M., and Nickum, J.G.	1964	The effect of Posthodiplostomum minimum upon the body weight of the bluegill. Prog. Fish Cult. 26:121-123.
Mahajan, C.L.	1978	Parasitization of Isoparorchis hypselobagri Billet in Channa punctatus Bloch. Curr. Sci. 47(21):835-836.
Mahajan, C.L., Agraval, N.K. John, M.J. and Katta, V.P.	1979	Effect of the digeneans, Isoparorchis hypselobagri, on an air breathing fish, Channa punctatus, with particular reference to bio-chemical and haematological changes. J. Fish Dis. 2(6):519-528
[illegible]	1 [illegible]	[illegible]estode Diseases of [illegible]arine [illegible]shes in: Symposium on Diseases of [illegible]shes and Shell fishes, ed. [illegible]. [illegible]hniszko, [illegible]merican [illegible]sheries Society, [illegible]ashington, p. 1[illegible]-2[illegible].
and Knapp, S.E.		poisoning trematode N. Salmincola to fish." In Snieszko, S.F.(edit.) Spl. Publs. Am. Fish Soc., No. 5, 209-217.
Molnar, K.	1980	A histological study on Ancylodiscoidosus in the sheat fish (Silurus glanis). Helminthologia, 17(2):117-126.
Nagasawa, R.K. and Egusa, S.	1981	Acanthocephalus lucidus. Van cleave (Acanthocephala : Echinorhynchidae). from cultured Rainbow trout Salmo gairdneri. Bull. Jap. Soc. Scient. Fish. 47(9):1153-1156.
Pickering, and Christie, P.	1980	Sexual differences in the Incidence and severity of Ectoparasitic infestation of the Brown trout, Salmo. trutta. Fish Biol. 16(6):669-683.

Prakash, A. and Adams, J.R.	1960	A histopathological study of the intestine lesions induced by *Echinorhynchus lageniformis* (Acanthocephala Echinorhynchidae) in the starly flounder. *Can. J. Zool.* 38(5):895-897
Qasim, S.Z. and Bhatt, V.S.	1966	The growth of the fresh water murrel, *Ophiocephalus punctatus* Bloch. *Hydrobiologia* 27:289-316.
Qayyum, A and Qasim, S.Z.	1964	Studies on the biology of some fresh water fishes Part I. *Ophiocephalus punctatus* Bloch. *J. Bombay Nat. Hist. Soc.* 61(7):74-98.
Robideau, T.R., and Self, J.T.	1953	A study of the effects of black and yellow grubs on *Lepomis cyanellus* and *L. humilis* in a natural environment. *Amer. Midl. Nat.* 50:402-406.
Radhakrishnan, S. and Nair, N.B.	1981	On Nybelinia Sp plerocercoid (Cestoda:Tentaculariidae) infection of the oesophagus of *Diodon hystrix* (Pisces:Diodontidae). *Proc. Indian Acad. Sc.* 90(3):281-289.
Rai, P.	1968	On the clinostomatid metacercariae in some of our edible fishes and remarks on the pathological significance. *Indian J. Anim. Sci.* 40(2):189-198.
Rao, K.H. and Rao, M.V.R.	1970	Seasonal occurrence of *Elongoparorchis pneumatis* (Hanumantha Rao, 1961) in the air bladder of the cat fish, *Arius thalassinus* Ruppell from Bay of Bengal. *Curr. Sci.* 39(24):562-563.

Ribelin, W.E. and Migaki, G.	1975	'The Pathology of Fishes'. The University of Wisconsin Press, Madison, Wisconsin.
Riis, R.C. Georgi, M.E. Leibovitz, L. and Smith, J.S.	1981	Ocular metacercarial infection of the Oyster toad fish, Opsanus tau (L). J. Fish Dis. 4(5):433-435.
Roberts, R.J.	1978	Fish Pathology. Bailliere Tindall, London.
Rodgers, L.J. and Burke, J.B.	1981	Seasonal Variation in the prevalence of "Red Spot" Disease in Estuarine Fish with particular reference to to the sea Mullet, Mugil cephalus. J. Fish Dis. 4(4):297-307.
Rogers, W.A.	1978	The principal parasitic diseases of warm water fishes. Mar. Fish Rev. 40(10):36.
Rydlo, M.	1975	Pathogenicity and transmission possibilities of fish pathogens Acanthocephala. Fisch umwelt. No.1, 105-112.
Saeed and Bilquees, F.M.	1971	P. magnum new Sp (Acanthocephala: Quadrigyridae) from the fish Wallago attu of Kalri Lake, West Pakistan. Pak. J. Zool. 3(2):221-223.
Sahay, U., Sinha, A. and Ghosh, A.K.	1971	On Davendrosentis garvai (Neoechinorhynchidea : Acanthocephala) from a fresh water Indian fish, Chipisoma garva (Ham). Ann. Parasit. hum. comp. 46:69-80.

Schmidt, G.D., Walley, H.D. and Wijek, D.S.	1974	Unusual pathology in a fish due to the acanthocephalan Acanthocephalis (Jacksoni Bullock) J. Parasit. 60(4):730-731.
Shariff, M. Richards, R.H. and Sommerville, C.	1980	The histopathology of acute and chronic infections of rainbow trout, Salmo gairdneri (Richardson), with eye flukes, Diplostomum Spp J. Fish Dis. 3(6):455-465.
Siddiqui A. and Nizami, W.A.	1982	Seasonal population dynamics of the metacercariae of Clinostomum complanatum (Trematoda:Digenea) in relation to sex of the host. Rivista Di Parassitologia 43(2):275-279.
Simms, B.T.	1933	Pathogenicity of metacercariae of N. Salmincola (Chapin) for fresh hosts. J. Parasit. 19:160.
Sircar, M. and Sinha, D.P.	1980	Histopathology of Lytocestus indicus infection in the fish, Clarias batrachus. Indian J. Anim. Res. 14(1):53-56.
Snedecor, G.W.	1959	Statistical Methods, Iowa:Iowa State College Press., Ames.
Snieszko, S.F.	1972	Progress in fish pathology in this century. Symp. Zool. Soc. Lond. 30:1-15.
Sommerville, C.	1981	A comparative study of the tissue response to invasion and encystment by Stephanochasmus baccatus (Nicoll, 1907) (Digenea : Acanthocolpidae) in four Sp. of flat fish. J. Fish Dis. 4(1):53-68.

Spell, R.D. and Summervelt, R.C.	1970	Life cycle of the white grub, Posthodiplostomum minimum (Maccallum, 1921 : Trematoda, Diplostomatidae), and observations on host-parasite relationships of the metacercariae in fish. In Snieszpo, S.F. (edit.) Spl. Publs., Am. Fish Soc., No.5, 218-229
Sultan, S. and Khan S.M.	1981	Length-weight relationship of Barbus stigma. Jour. Sci. Res. 3(2):101.
Swarup, H., Pathak, S.C. and Arora, S.	1981	Microtomy. In : Laboratory Techniques in Modern Biology. by Usha Raj Kumar for Kalyani Publishers, New Delhi, 189pp.
Sweeting, R.A.	1977	Studies on Ligula intestinalis some aspects of the pathology in the second intermediate host. J. Fish Biol. 10(1):43-50
Wabuke and Bunoti, M.A.N.	1980	The prevalence and pathology of the cestode Polyonchobothrium clarias (Woodland, 1925) in the teleost Clarias mossambicus. J. Fish Dis. 3(3):223-230.
Williams, H.H.	1967	Helminth diseases of fish. Helminthol Abstr. 36:261-295.
Wood, E.M. and Yasutake, W.T.	1956	Histopathology of fish II. The salmon poisoning fluke. Prog. Fish Cult. 18:22-25.
Ward, H.B. and Mueller, J.F.	1926	A new popeye disease of trout fry. Arch Schiffs-4. Tropen. Hyg. 30:602-609.